

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Gladwin *et al.*

Application No. 10/563,683

Filed: October 4, 2006

Confirmation No. 3225

For: USE OF NITRITE SALTS FOR THE
TREATMENT OF CARDIOVASCULAR
CONDITIONS

SUBMITTED VIA EFS

Examiner: Anna Pagonakis

Art Unit: 1614

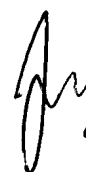
Attorney Reference No. 4239-67618-07

COMMISSIONER FOR PATENTS
SUBMITTED VIA ELECTRONIC FILING SYSTEM

DECLARATION OF DR. JON LUNDBERG UNDER 37 C.F.R. § 1.132

I, Jon Lundberg, Ph.D., declare as follows:

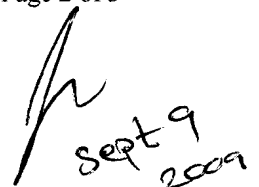
1. I have no financial interest in the above referenced patent application and I am not a listed inventor of the invention disclosed in the above referenced patent application.
2. A copy of my *curriculum vitae* is attached hereto as **Exhibit A**. At present, I hold a position as Professor in the Department of Physiology and Pharmacology at the Karolinska Institute in Stockholm, Sweden. I have had more than 15 years of experience in research including work on the physiological effects of nitric oxide and inorganic nitrate and nitrite and particularly the effects of these molecules on vascular tone. I have published over 100 scientific articles in scientific journals and books. By virtue of my education, training, and professional experience, I am knowledgeable about nitric oxide donors, the physiology and biology of vasodilatation, and the effects of various compounds on vasodilatation. I am considered one of the world experts in the study of nitrate, nitrite and nitric oxide, and recently hosted an international meeting on nitrite biology in Stockholm Sweden.
3. I supervised the studies published in Modin *et al.*, *Acta Physiol Scand.*, 171:9-16, 2001 (attached hereto as **Exhibit B**), a reference I co-authored. The data presented in this

 **Sept 9 2009**

reference teaches that inorganic nitrite must be in a slightly acidic environment (that is, “acidified”) to function effectively as a vasodilator. The experimental model we used in this publication did not include the regulatory factors present in blood, such as hemoglobin in red blood cells, that are known to inhibit the effects of nitric oxide and nitric oxide donor medications. Because of this, our finding that concentrations of non-acidified (pH 7.45) inorganic nitrite greater than 25 micromolar concentration cause vasodilation of excised rat aorta were not considered predictive of whether or not similar concentrations of inorganic nitrite would cause vasodilation under non-acidic/non-hypoxic physiological conditions *in vivo*. I do not believe that the data presented in the Modin *et al.* reference teaches that non-acidified sodium nitrite is a vasodilator at concentrations of 25 μM or less, *in vitro* or *in vivo*. Reading Figure 2 of Modin *et al.*, I do not believe that inorganic nitrite, when applied in a neutral buffer under the prevailing experimental conditions, is an effective vasodilator of isolated segments of rat aorta at concentrations of 25 μM or less.

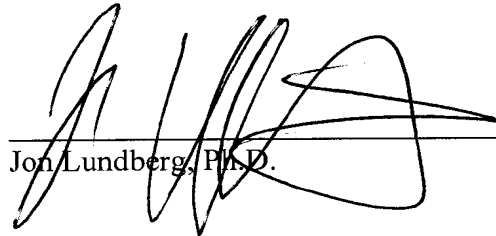
4. Although we published that inorganic nitrite caused vasodilation of isolated segments of rat aorta *in vitro* at neutral pH with an EC_{50} value of 200 μM , the overwhelming evidence in the scientific literature prior to October 14, 2003, was that near physiological concentrations, non-acidified sodium nitrite was vasodilator-inactive under normoxic conditions – particularly *in vivo*. Prior to October 14, 2003 the conventional wisdom among the majority of scientists in the nitric oxide field was that inorganic nitrite was an inert oxidation product of nitric oxide metabolism. This is exemplified by Lauer *et al.*, *Proc. Natl. Acad. Sci. USA*, 98:12814-12819, 2001 (attached hereto as **Exhibit C**), a reference I have read and am familiar with, which teaches that no vasodilation occurs at venous plasma nitrite concentrations of 130 μM (see page 12816, column 2, last paragraph) and that physiological levels of nitrite are vasodilator-inactive (see the abstract). It was thus therefore very surprising to most scientists that the studies of Cosby *et al.* (*Nature Med* 9(12):1498-1505, 2003) showed that nitrite was a vasodilating agent at or near physiological concentrations. In fact, Cosby *et al.* was met with wide skepticism until the results were reproduced later by a number of other laboratories.

5. All statements made herein and of my own knowledge are true and all statements made on information are believed to be true; and further, these statements were made with the

A handwritten signature, possibly 'H', is written above the date 'Sept 9 2009'.

knowledge that willful false statements and like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

Date September 9TH
2009


Jon Lundberg, Ph.D.